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Table 2. The Major Human Polymorphic *CYP2D6* Alleles and their Global Distribution. For a Complete List, See *http://www.imm.ki.se/cypalleles/cyp2d6.htm*

Major Variant Alleles	Mutation	Consequence	Allele Frequencies (%)			
			Caucasians	Asians	Black Africans	Ethiopians and Saudi Arabians
*CYP2D6*2xn*	gene duplication/ multiduplication	increased enzyme activity	1–5	0–2	2	10–16
*CYP2D6*4*	defective splicing	inactive enzyme	12–21	1	2	1–4
*CYP2D6*5*	gene deletion	no enzyme	2–7	6	4	1–3
*CYP2D6*10*	P34S, S486T	unstable enzyme	1–2	51	6	3–9
*CYP2D6*17*	T107I, R296C, S486T	reduced affinity for substrates	0	ND	34	3–9

ND: not determined.

PM phenotype predispose for diseases besides melanoma, lung-, breast-, anogenital-, basal cell-aerodigestive tract, oral-, prostate-, pancreatic-, and bladder cancer, also with parkinsonism, Alzheimers disease, optic neuropathy, tremor, hair color, neuroleptic malignant syndrome, smoking behavior, opiate dependence, tardive dyskinesia, tremor, hematologic neoplasias, and Lewy body disease, and so forth, but no established relation has hitherto been found.

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DECORIN

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Decorin is a member of the growing family of the extracellular small leucine-rich proteoglycans (SLRPs) that includes biglycan, fibromodulin, lumican, PRELP (proline arginine-rich end leucine-rich repeat protein), keratocan, osteoadherin, epiphycan, opticin, and osteoglycin. It is a ubiquitous component of extracellular matrices synthesized by most cells of mesenchymal origin. The name decorin (also called *PG40, PG-2, PG-II, PG-S2, CS-PGII*, and *DS-PG II*) was coined from its ability to coat or decorate the surface of the collagen fibrils. In doing so, it may control the assembly and structure of the collagen fibrils. Decorin also binds to the transforming growth factor-β (TGF-β), thereby modulating its activity. The human gene is located on chromosome 12q21.3 and is composed of eight exons, the first of which is alternatively spliced from different 5′ genomic sequences. The decorin promoter contains a single regulatory element with a binding site for the transcription factor AP-1 that mediates the inhibitory effect of tumor necrosis factor-α (TNF-α) and the stimulatory effect of interleukin 1 (IL-1) on decorin expression. Decorin controls cell growth and suppresses the malignant phenotype of a tumor cell through EGF (epidermal growth factor)-activation of the externally regulated kinase (ERK)1,2 signaling pathway and the upregulation of $p21^{WAF1/CIP1}$. No genetic mutations for the *decorin* gene have been described to date. However, aberrant expression of decorin has been reported in numerous connective tissue disorders such as carbohydrate-deficient glycoprotein syndrome, Ehlers-Danlos syndrome, Marfan syndrome, osteogenesis imperfecta, pachydermoperistosis, and scleroderma.

PROTEIN STRUCTURE AND FUNCTION

The decorin core protein is approximately 38 kDa and consists of three regions: an amino-terminal region with a single covalently bound glycosaminoglycan (GAG), a central domain, composed of 10 leucine-rich repeats (LRRs) bearing two to three *N*-oligosaccharides that is flanked by cysteine-rich clusters, and a carboxyl-terminal region (Fig. 1) (1). The GAG is chondroitin sulfate in bone and dermatan sulfate in most soft tissues. The LRRs of the central domain are 24–amino acids long with Asparagine (N) and Leucine (L) residues in conserved positions LxxLxLxxNxLx (x = variable residue). Decorin also contains a 16–amino acid hydrophobic signal peptide for secretion (pre-peptide), followed by a 14–amino acid propeptide. The propeptide is cleaved under conditions that are tissue-specific and age-dependent (2). The biological

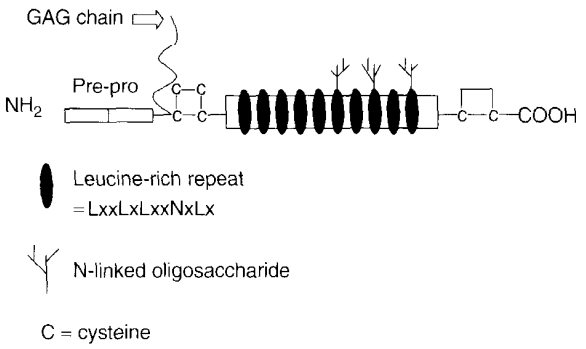

Figure 1. Decorin structure.

significance of this cleavage is not known. After digestion of the GAG with chondroitinase ABC, decorin (lacking the propeptide) is a doublet band (explained by the presence of two or three N-oligosaccharides on the protein core) of Mr approximately 45 and 47 kDa on a SDS-PAGE gel (SDS polyacrylamide gel electrophoresis).

Decorin binds to various proteins including collagen type I, II, III, V, VI, and XIV, C1q complement factor, and TGF$_\beta$. Its binding to fibronectin and thrombospondin, two extracellular matrix proteins, inhibits cell adhesion (3).

Decorin binds to the d-band of type I collagen mainly through its sixth leucine-rich repeat (4). Molecular modeling and electron microscopy predict that decorin has a horseshoe shape with β-sheets forming its inner concave surface and α-helices making up the outer convex surface, the GAG chain, and the three *N*-oligosaccharides being all located on the outer surface (5). The horseshoe-shaped decorin can theoretically accommodate a single collagen triple helix. The direct binding of decorin is believed to control the assembly and structure of collagen fibrils. This is supported by the observation that decorin-deficient mice exhibit fragile skin due to abnormally coarse and irregular collagen fibrils (6). Decorin also links low density lipoproteins (LDL) to collagen of the arterial intima, that may be an important step towards the initiation of atherosclerosis (7).

Decorin controls the activity of TGF-β a multifunctional cytokine involved in cell proliferation and differentiation, by sequestering it into extracellular matrix and preventing it from interacting with its cell surface receptors (8). This regulatory effect of decorin on the TGF-β activity could be used therapeutically. Injection of decorin into rats with experimental glomerulonephritis, a fibrotic disease characterized by accumulation of extracellular matrix, prevents matrix deposition (3).

GENE STRUCTURE, REGULATION AND EXPRESSION

The human *decorin* gene (GeneBank accession numbers L01125–31 and M98262–3) is located on chromosome 12q21.3 close to the *lumican* and *epiphycan* (also called DSPG3) genes, two other SLRPs (9). The gene is over 38 kilobases (kb) long and composed of seven introns and eight exons. The exons do not appear to encode discrete cysteine clusters or tandem repeat units but, interestingly, are identical in length and position to the *biglycan* gene (see *biglycan* for a discussion on the consequences of this similarity). However, unlike the *biglycan* gene, two untranslated, alternatively spliced leader exons (Ia and Ib) were discovered in the 5′ untranslated region of the *decorin* gene (10). The upstream region of exon Ib contains a 150-base pair homopurine and homopyrimidine segment involved in the regulation of gene transcription (11). It also contains a regulatory element, that mediates the inhibitory effect of TNFα and the stimulatory effect of IL-1 on *decorin* gene expression (12). This regulatory element contains a binding site for AP-1, a transcription factor family formed of dimers of gene products of the *Fos* and *Jun* families of oncogenes. Overexpression of *c-jun* was shown to result in the inhibition of the decorin promoter activity, whereas blocking of *c-jun* expression resulted in enhanced stimulation to IL-1 and reverse responsiveness to TNF-α indicating that the c-Jun–AP1 complexes are potent inhibitors of decorin transcription.

DECORIN AND DISEASES

Decorin belongs to a class of molecules involved in growth suppression called quiescins. It is upregulated in quiescent fibroblasts and rarely expressed in tumor cells. Ectopic expression of decorin results in the suppression of the malignant phenotype in a large variety of human tumor cell lines (13). In the case of A431 colon carcinoma cells, the secreted decorin core protein binds to the epidermal growth factor (EGF) receptor leading to the activation of the mitogen-activated protein (MAP) kinase (ERK)1,2 signaling pathway (14) and to the upregulation of $p21^{WAF1\ (CIP1)}$, an inhibitor of cyclin-dependent kinases whose activity is required for cell cycle progression (13). Interestingly, the EGF-activation of the same MAP kinase signaling pathway in fibroblasts also downregulates the decorin expression by these cells indicating the ability of decorin to autoregulate its own expression (15). Recently, decorin was also shown to be a potent cell migration inhibitor (16). Although decorin suppresses growth of tumor cells and stops cell migration, decorin-deficient mice do not develop spontaneous tumors. However, the lack of decorin accelerates lymphoma tumorigenesis in mice deficient in *p53*, an established tumor-suppressor gene (17).

Although no specific human disease has been unambiguously ascribed to mutations in decorin, aberrant expression of decorin has been reported in numerous connective tissue disorders (carbohydrate-deficient glycoprotein syndrome, Ehlers-Danlos syndrome, Marfan syndrome, pachydermoperistosis, and scleroderma) (3). In all these cases, the aberrant expression of decorin is a secondary effect of a primary mutation located on another gene. Finally, in osteogenesis imperfecta patients, absence of decorin was reported to result in a more severe phenotype.

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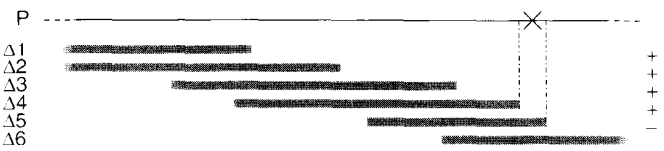

Figure 1. Deletion mapping of a new point mutation in a simple organism. The point mutant (P) is crossed to a panel of deletion mutants (Δ1–6) where the extent of deletion is known, shown by thick bar. If recombination yields a wild-type phenotype (+) then the point mutant lies outside the deletion. If recombination does not yield a wild-type phenotype (–) then the point mutant lies within the deletion. Comparison of a series of overlapping deletions can accurately map the point mutation. This can be used to map a gene relative to other genes or map the position of an allele within a gene (X). Similar logic is applied to panels of deletion mutants used in several strategies for the mapping of human genes.

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DELETION MAPPING

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Deletion mapping is the use of deletion mutants for the physical mapping of genes, either in relation to other genes in the genome or in terms of genetic fine structure. The term covers a range of techniques with resolution ranging from megabase pairs to single nucleotides. Deletion mapping originated as a technique to map the precise position of point mutations in phage genomes (1,2). The principle of deletion mapping reflects the fact that recombination between two mutant phages can produce a wild type if the parent strains have mutations at different positions in the genome. If one of the strains carries a deletion and the other a point mutation, wild types can arise only if the point mutation lies outside the deletion. Uncharacterized point mutations can therefore be mapped by crossing to a panel of characterized deletion mutants (i.e., where the extent of each deletion is known) as shown in Figure 1. This type of deletion mapping has been applied to many genetically amenable organisms, including yeast and *Drosophila*, although it is unsuitable for humans. However, deletions can be used in a number of different ways to determine the position of human genes, as discussed later.

CORRELATION BETWEEN CYTOGENETIC DELETION AND PHENOTYPE

Many human diseases are caused by cytogenetic deletions, that is, deletions that are apparent at the cytogenetic level through the absence of one or more chromosome bands. In such cases, it can be assumed that critical genes map in the region spanned by the deletion, thus providing a rough map position. However, even small visible chromosomal deletions may correspond to several megabases of lost DNA and may affect a series of linked genes. Microdeletions affect fewer genes, but may be more difficult to detect through cytogenetic analysis alone (some molecular methods for detecting microdeletions are discussed later). Within the region spanned by a deletion, some genes may play a major role in the disease and others only minor roles, and it may therefore be difficult to identify the genes involved. However, it may be possible to narrow down the position of major disease loci within the deletion by looking at the effects of smaller deletions within or overlapping the same region. For example, Turner's syndrome results from the deletion of an entire X-chromosome in females, but certain aspects of the syndrome are seen in patients with smaller deletions. Because most X-linked loci are subject to dosage compensation in the female, the different features of Turner's syndrome result from haploin sufficiency at a small number of loci. The analysis of overlapping deletions on Xp has revealed candidate critical regions for different aspects of Turner's syndrome, including the *SHOX* locus, where haploinsufficiency may cause the characteristic short stature (3).

For X-linked deletions, disease phenotypes in males result from total loss of gene function and are often revealed as contiguous gene syndromes where each gene has a distinct effect on the phenotype. Conversely, autosomal deletions cause dosage effects, and may only involve a few loci that show haploinsufficiency among other loci that show mild dosage effects or no impairment of function at all. It can therefore be quite difficult to track down the true disease-causing genes within an autosomal deletion. Classic autosomal microdeletion syndromes that are largely uncharacterized at the gene level include Cri-du-chat syndrome (del 5p15.2–3) and Wolf-Hirschhorn syndrome (del 4p16.3). Although it can be assumed that important genes map within each deletion, candidates are only beginning to be identified. For example, recent investigations have identified a number of putative genes in the Cri-du-chat critical region (4,5), but only a few, such as the gene-encoding semaphorin F, have been linked to the disease phenotype (6). In the Wolf-Hirschhorn critical region, a single gene encoding a calcium-binding protein has been identified, which may be responsible for some of the neurological symptoms of this syndrome (7). In other diseases, candidate genes within the deletion have been well characterized in terms of their role in the clinical phenotype. For example, individuals